

Multiple Strategy Bio-Detection Sensor Platforms

Made from Carbon and Polymer Materials

Interim Report

ONR Award No. N00014-03-1-0893

for the work performed Jan 01, 2005 – June 30, 2005

**Dr. Ryan Giedd, PI
Dr. Kartik Ghosh, Co-PI
Mr. Matt Curry, Co-PI
Mr. Rishi Patel, Co-PI
Dr. Paul Durham, Co-PI
Dr. Pat Kinlen, PI of Crosslink Polymer Research Inc. Subaward
Dr. Xuliang Han, PI of Brewer Science Inc. Subaward**

**Center for Applied Science & Engineering
Southwest Missouri State University
901 South National Avenue
Springfield MO, 65804**

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

Table of Contents

	Page No.
I. Introduction.....	3
II. Description of Technical Research Progress	
a. Active Sense and Release	3
i. Polymer based release systems on CPMEMS platform.....	3
ii. Actual drug release	9
b. Progress on Self Assembled Protein Layer.....	12
i. Nitrocellulose study	12
ii. Functionalizing of Au/PSA.....	14
c. Update on Unity 400 Heat Dissolvable Polymer	18
III. Conclusions	
a. Accomplishments and Future Work	22

I. Introduction

This interim progress report will cover the progress to date on the development of a multiple strategy bio-detection sensor platform made from carbon and polymer based microelectromechanical systems (CPMEMS). Progress since the last interim report has involved the development of a sense and release experiment, and further a proof of concept of actual drug release on a release experiment. Other points of progress include studies on alternate methods of developing the self assembled protein layers, specifically utilizing a nitrocellulose thin film layers or a silane functionalizing monolayer to bind proteins, antibodies or oligonucleotides to the CPMEMS sensor platform. Progress on an alternate sacrificial layer (Unity 400) will also be discussed in this report.

II. Description of Technical Research Progress

a. Active Sense and Release

i. Polymer based release system on a CPMEMS platform

Crosslink has been investigating the development of active sense and release experiments for incorporation into the broadband platform. In the last interim report, a demonstration of small carbon-based electrodes that were developed and prepared via Crosslink's in-house printing methods was established. Polypyrrole/phenolred was deposited on the carbon electrodes and films of different thickness were made utilizing an electropolymerization method. Crosslink developed an analytical method for measuring the released dopant (phenolred) and obtained the release profiles of PPY/phenolred in solution at varying conditions. The results showed the release mechanism under an applied voltage is a function of the film thickness with burst release changing to diffusion controlled release with increasing film thickness.

The method to deposit PPY/phenolred based films on the surface of the small carbon electrode cannot simply be applied to a microelectrode without substantial modifications. Figure 1 shows a schematic system for depositing PPY/Dopant film on the surface of microelectrodes prepared via photolithography on a silicon wafer. A bi-potentiostat from GAMRY was employed for the electro-polymerization experiments using either chronoamperometry or cyclic voltammetry. MEMS electrodes made with Au were fabricated on a silicon wafer via photolithography. There are over 120 microelectrodes on each silicon chip and each square microelectrode has a size of 125 micron². The reference electrode was a silver wire and a gold wire served as the counter electrode. The growth of the film was followed via optical microscopy.

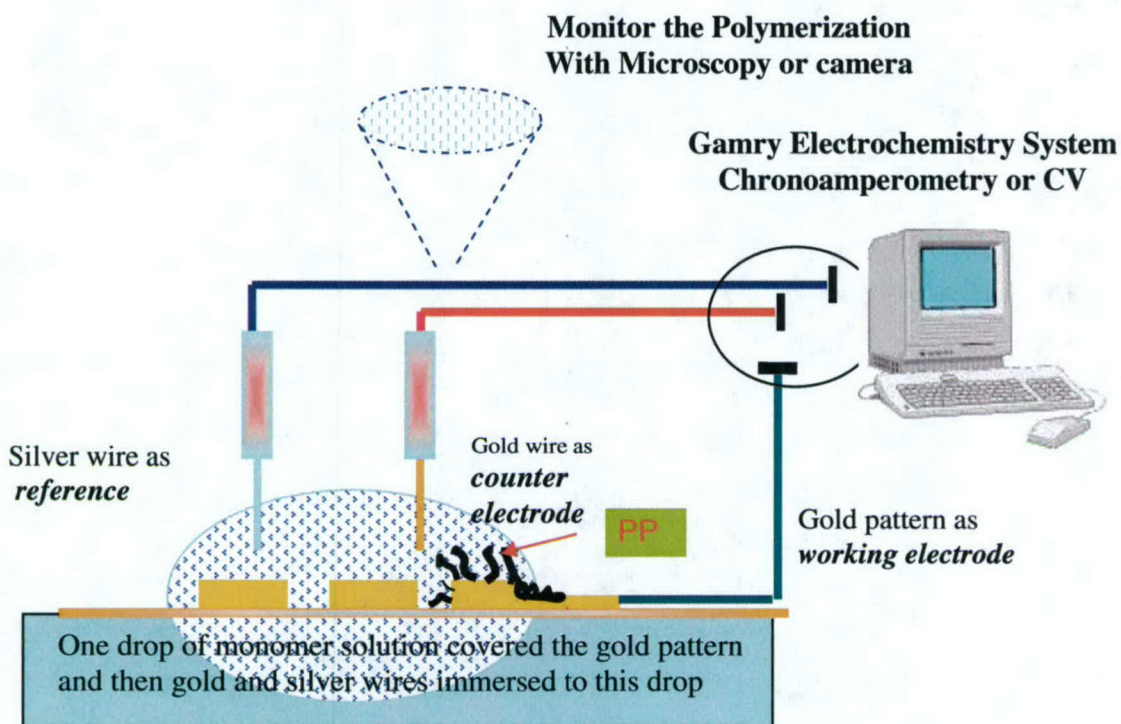


Figure 1: Electrochemical polymerization to Deposit PPY/Phenolred on Microelectrode

The microelectrodes used for the release experiments allowed for only a small volume of the solution to be utilized in the reaction. In a typical polymerization experiment, a drop of solution (less than 0.2 ml) containing pyrrole (0.1 M) and dopant molecule (0.1 M) was added to the surface with a syringe to cover a selected electrode. The tips of the reference and the counter electrodes were then immersed into the solution. A constant voltage of 0.8 V was applied (chronoamperometry) to the electrode. When the polymerization stopped, any unreacted solution was removed and the deposited polymer was then rinsed with water 3 times to remove the unreacted monomer and any dopant molecule on the surface. After air drying, the coated microelectrodes were examined by either scanning electron microscopy (SEM) or optical microscopy. In these studies, the phenolred dopant molecule was released to demonstrate a proof of concept.

SEM allows the observation of the deposition of PPY/dopant film on the microelectrode. Figure 2a shows that PPY/chloride film only formed on the surface of those two selected microelectrodes whereas other microelectrodes remained intact. No film grew in the gap between the two adjacent microelectrodes although the gap was covered with the reaction solution. Figure 2b shows the enlarged SEM image of the first microelectrode. The film surface was rough with additional substructures formed in the film. Figure 2c shows the enlarged SEM image of the second microelectrode and again the rough PPY/Chloride film formed on the electrode. The electro-polymerization time

for the second microelectrode was 4800 seconds, compared to 3600 seconds for the first. In fact, the textures of the films on both microelectrodes show similar features to previous literature reports of PPY/Chloride film deposited on larger electrodes. Identical polymerization conditions were used to make PPY/phenolred on the microelectrodes.

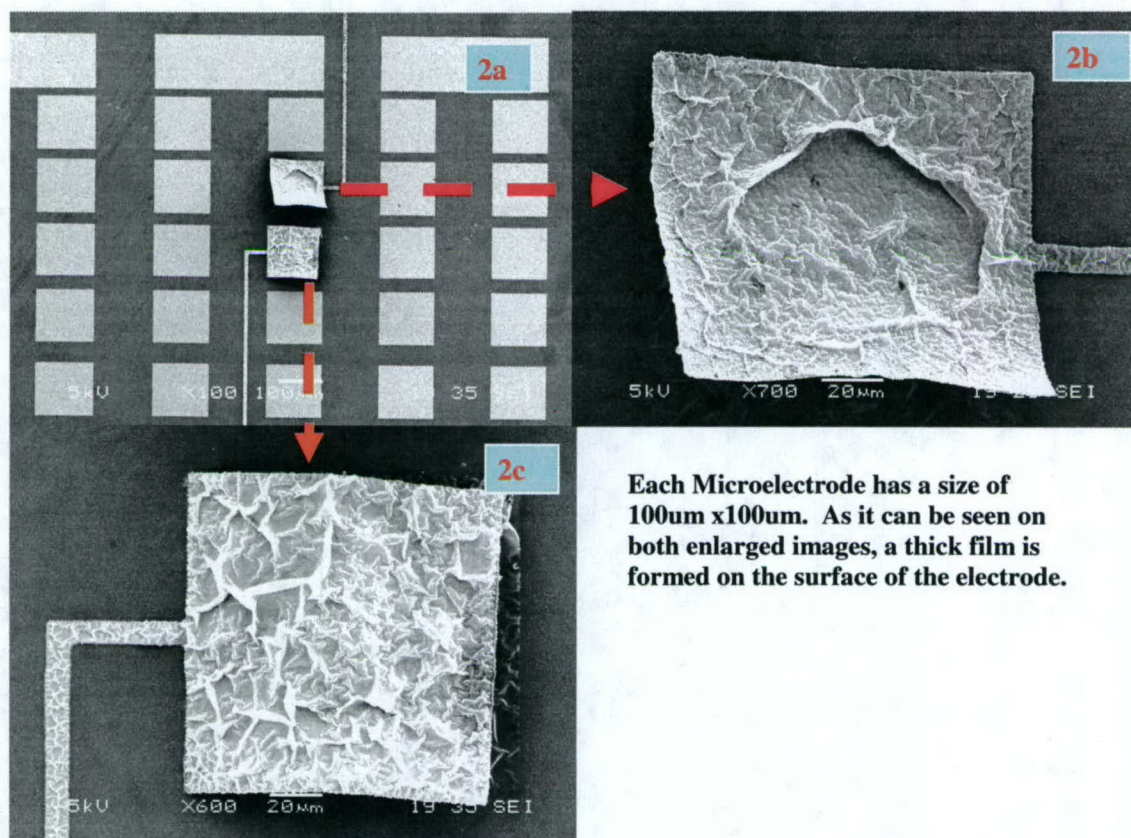


Figure 2: SEM Images of the Deposition of PPY/Chloride on the Surface of Two Selected Microelectrodes

Figure 3a-3d shows the images of the growth of PPY/phenolred on two microelectrodes. As shown in the center of cycle in Figure 3b, the red spot indicates the growth of PPY/phenol red film on the first microelectrode. As the polymerization proceeds, PPY/phenol red film grew on the second microelectrode as indicated by the appearance of the second red spot in Figure 3c and 3d.

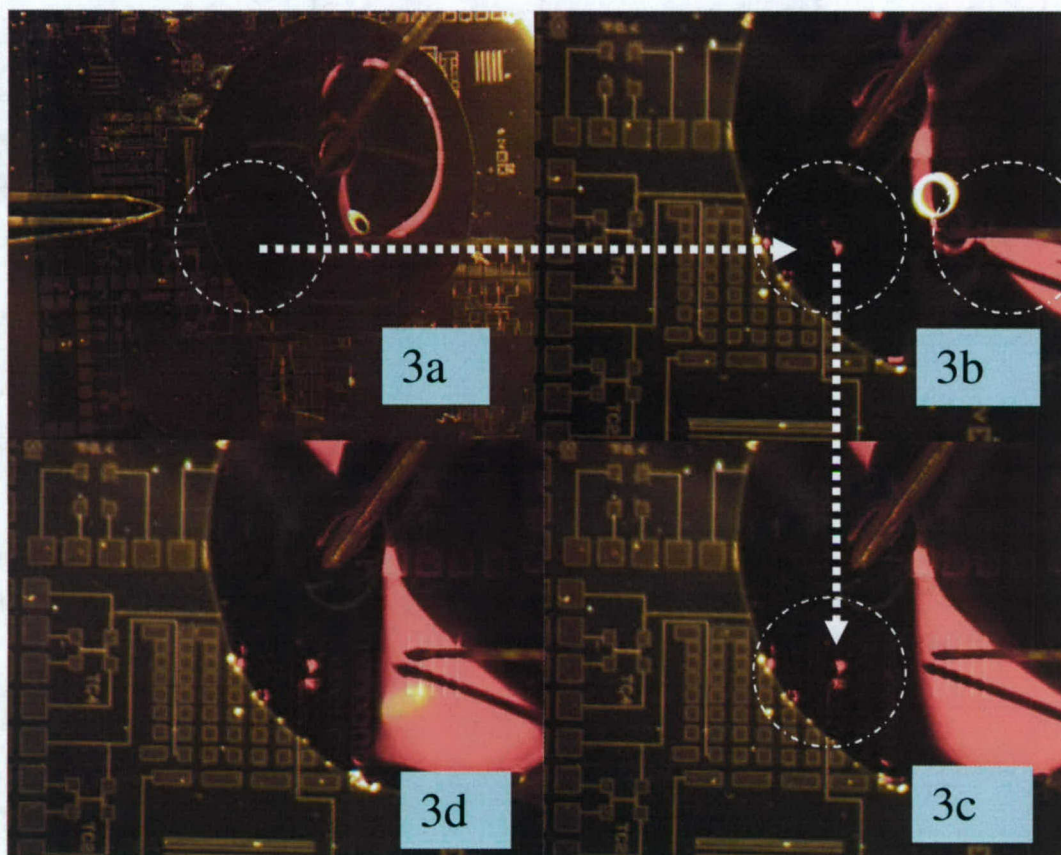


Figure 3: Observation of the Growth of PPY/Phenolred on the Surface of two Microelectrodes

In another typical experiment, phenolred was employed as a dopant molecule replacing chronoamperometry with cyclic voltammetry (CV) to polymerize pyrrole while other parameters remained the same. The “cell” was scanned in a cycle from -0.5V to 0.98V to -0.5V with a scan rate of 100mV/s. In theory, each CV scan polymerizes a thin layer of PPY/Phenolred film on the working electrode. The number of scan cycles controls the thickness of the film. After the experiment was complete, the film on the microelectrode was rinsed with water to remove the unused reagents and air dried. Using SEM, the film surface morphology was then monitored.

Figure 4a to 4d shows PPY/phenolred film formation on the microelectrodes. Only vertical growth of the PPY/phenolred film was observed. No lateral film growth was observed even when the scan number was increased to 72. As the scan number increased from 16 to 72, the film thickness increased accordingly. As shown in both figure 4a and 4b, smooth films were deposited on the microelectrode surface without any wrinkles forming in the film after 32 scans. However, the wrinkles were observed in the film when the scan number increased to 48 from 32 as shown in Figure 4c. Figure 4d shows that increasing scans beyond 48 results in even more wrinkles in PPY/phenolred film. In brief, PPY/phenolred films made from the CV method appeared smoother than those made from either the chronopotentiometry or the chronoamperometry method.

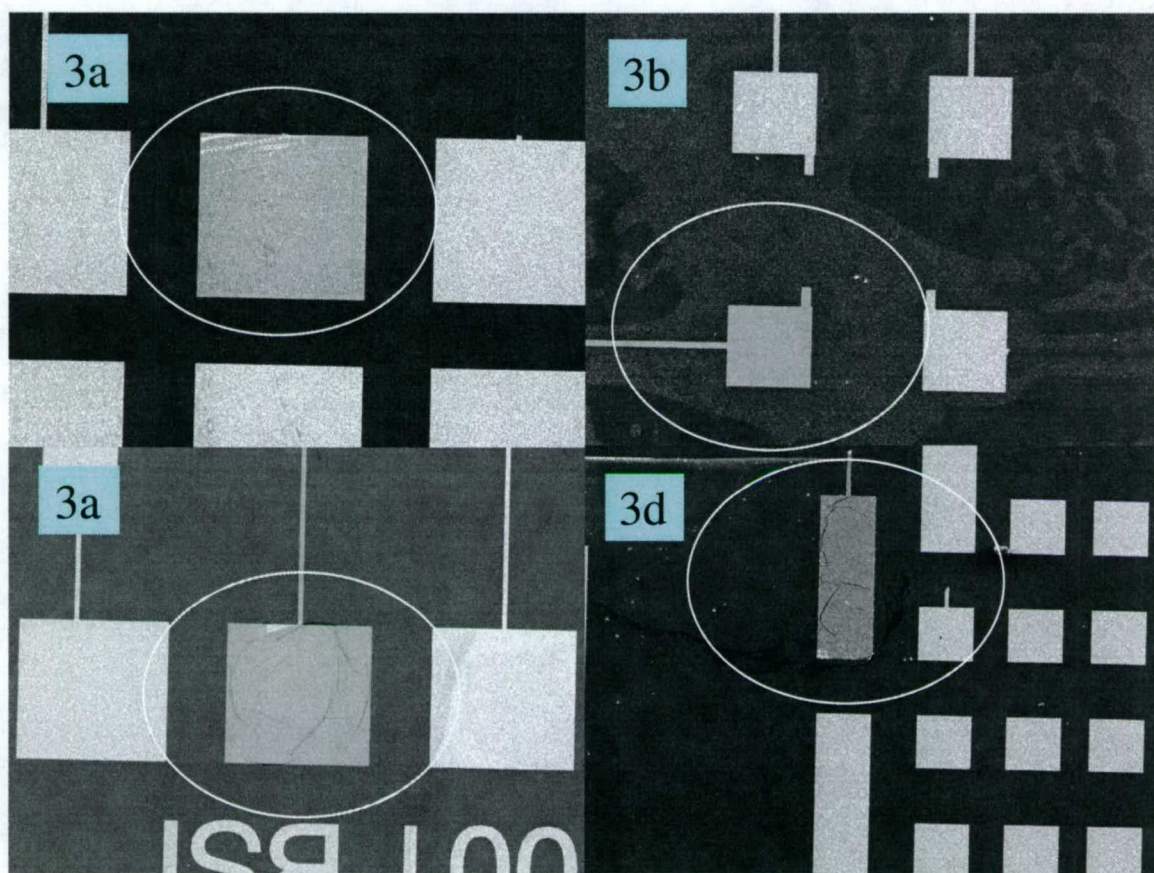


Figure 4: As the CV cycles increase, the film thickness increases. The thin PPY/phenolred film can be seen through

The release of Phenolred from PPY/phenolred on these microelectrodes was observed. The release of phenolred in solution from a PPY/phenolred film on a gold wire with a diameter of 100 microns was first measured. The size of the film on this gold wire is intermediate between that of the screen-printed carbon electrode but larger than that on the gold microelectrodes. The parameters or the protocol obtained from this release study should be better fit for the release study on the microelectrodes. A PPY/phenolred film was formed on the surface of a gold wire using chronoamperometry with a constant voltage of 0.8V for 3600 sec. The coated gold wire was rinsed with water and air dried. Figure 5 shows the electrochemical apparatus for measuring the release of phenolred. As shown in Figure 5b, a pink color associated with the released phenolred was observed after -1v was applied to the electrode. Figure 5c and 5d shows the continuous release of the phenolred in solution. Work was then conducted on applying a similar system to observe the release of phenolred from the microelectrodes. The release of phenolred was easily observed from the microelectrode using an optical microscope and visual observation. Unfortunately, the existing digital camera on the optical microscope being utilized is not sufficiently sensitive to this color change. More phenolred could be released by increasing the thickness of PPY/phenol red film.

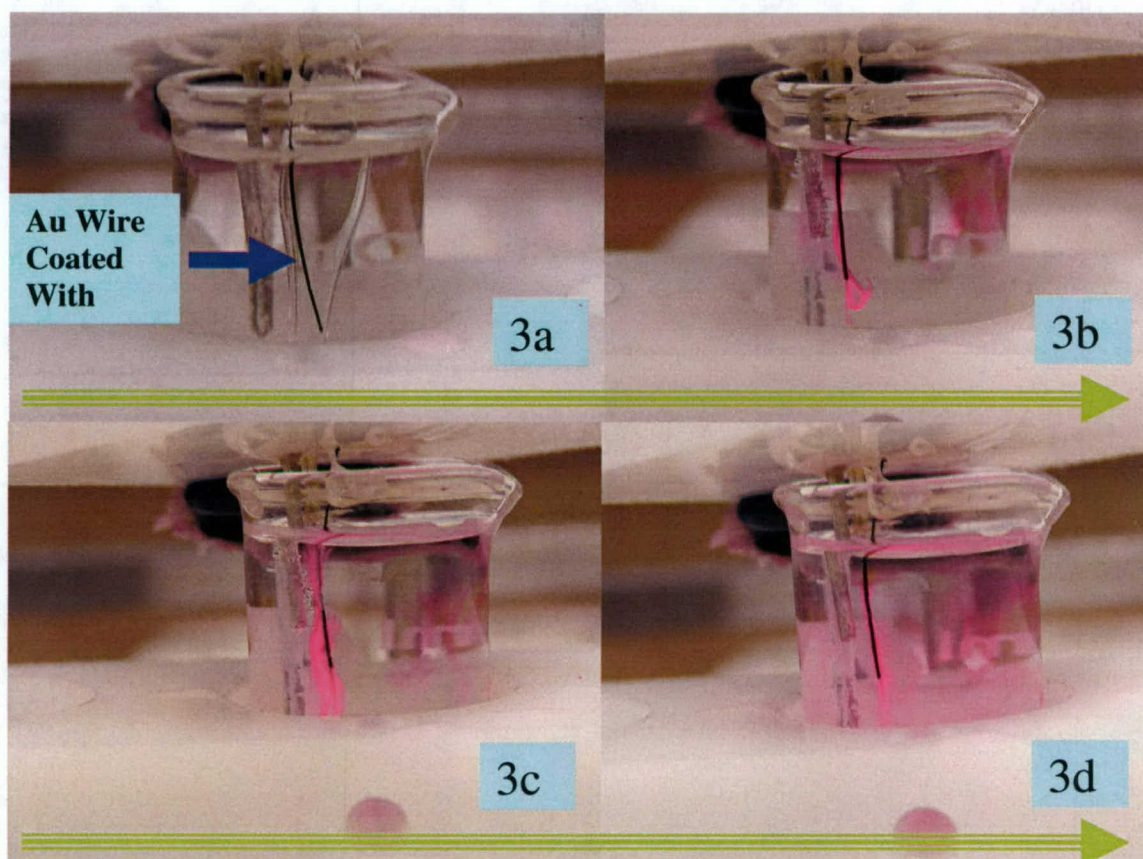


Figure 5: The Release of Phenol red from PPY/Phenolred on a Gold (Au) wire in a solution

Figure 6 displays a new design for future controlled release devices on a chip. The size of the device can be in the micron scale. The size of each microelectrode is variable. Each microelectrode (1-8 or a-g) can be coated with a PPY/dopant film via our electropolymerization method and ideally the dopant from each microelectrode can be released individually when a voltage is applied.

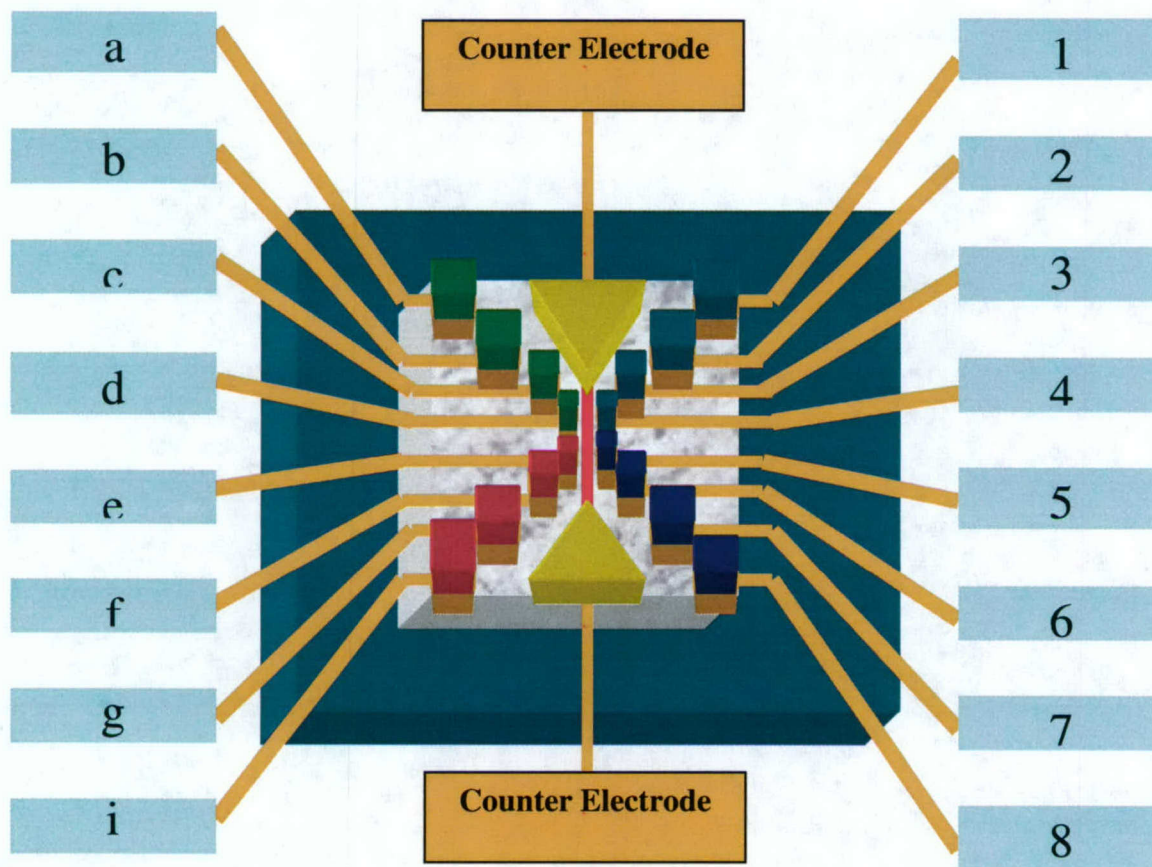


Figure 6: A Configuration of Multi Microelectrodes on a chip

ii. Actual drug release

The next step in the sense and release experiment employed ampicillin (amp), an antibiotic, as a model biological active dopant. Ampicillin is widely used in microbiological studies and a host of microbes have been engineered to be resistant or sensitive to ampicillin. Ampicillin is less stable than phenolred, and it lacks a convenient chromophore for spectrophotometer detection.

Polypyrrole/ampicillin films were prepared on the surface of the screen-printed carbon electrodes using a GAMRY potentiostat and chronopotentiometry. During electropolymerization, the applied electric current ranged from 0.1 mA to 1 mA and the concentrations of pyrrole and the dopant were kept at 0.1 M, respectively. The reaction time ranged from 400 to 4000 seconds. After the reaction was stopped, the PPY/Amp film on the carbon electrode was washed with water and air dried. Figure 7 shows the UV-Visible spectrum of sodium ampicillin in tris-buffered saline recorded with a UV-Visible spectrometer (model PC-1650C) from Shimadzu. The spectrum contains a peak absorption maximum at 210 nm. An analytical method for measuring the released

ampicillin was developed using the absorbance at 210 nm. Figure 8 displays the working curve of absorbance versus concentration giving a straight line passing through the origin.

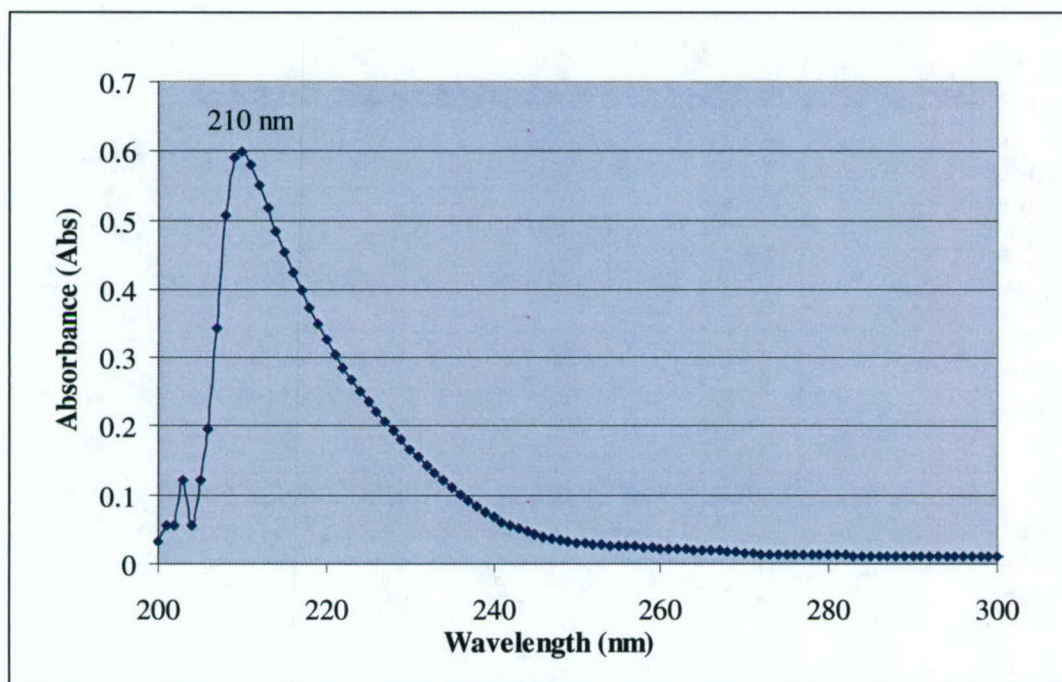


Figure 7: UV-Visible Spectrum of Sodium Ampicillin in Tri-Buffered Saline

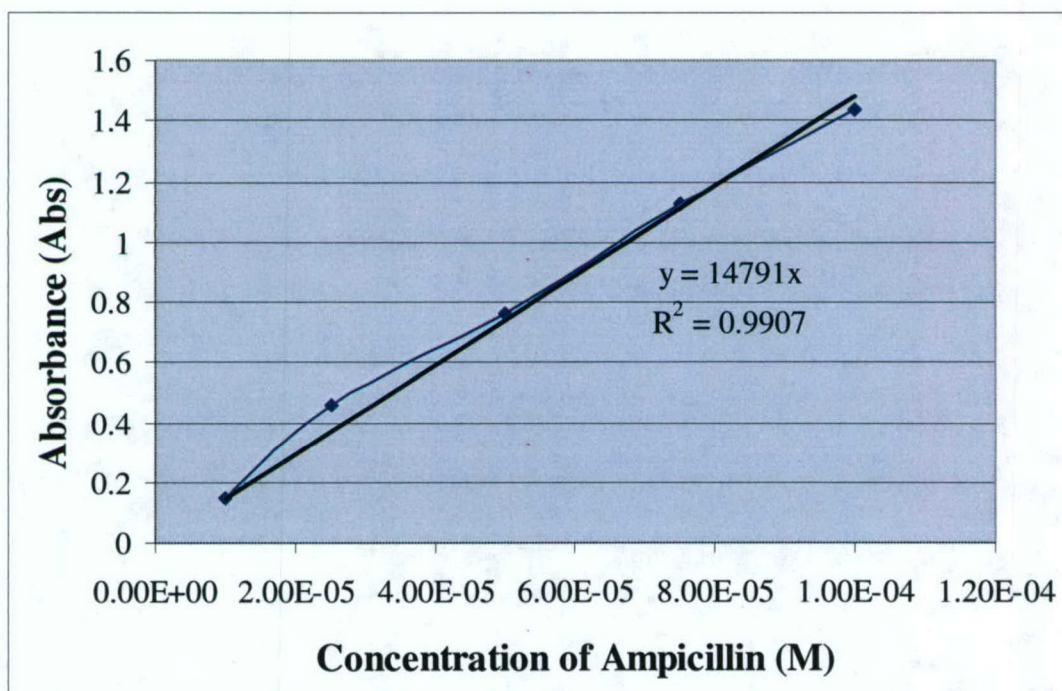


Figure 8: The Working Curve for Ampicillin in Tris-Buffered Saline

Release profiles of ampicillin from the PPY/amp film in solution was obtained by following the increase in absorbance at 210 nm. Figure 9 shows a release profile of ampicillin from PPY/amp. The PPY/amp was deposited on the surface of a carbon electrode at a constant current of 1 mA for 4000s. By applying a potential of -1 V, most of the amp was released after 10-15 minutes suggesting burst release of amp in this application. We also determined that amp slowly releases from the doped PPY film even in the absence of an applied potential.

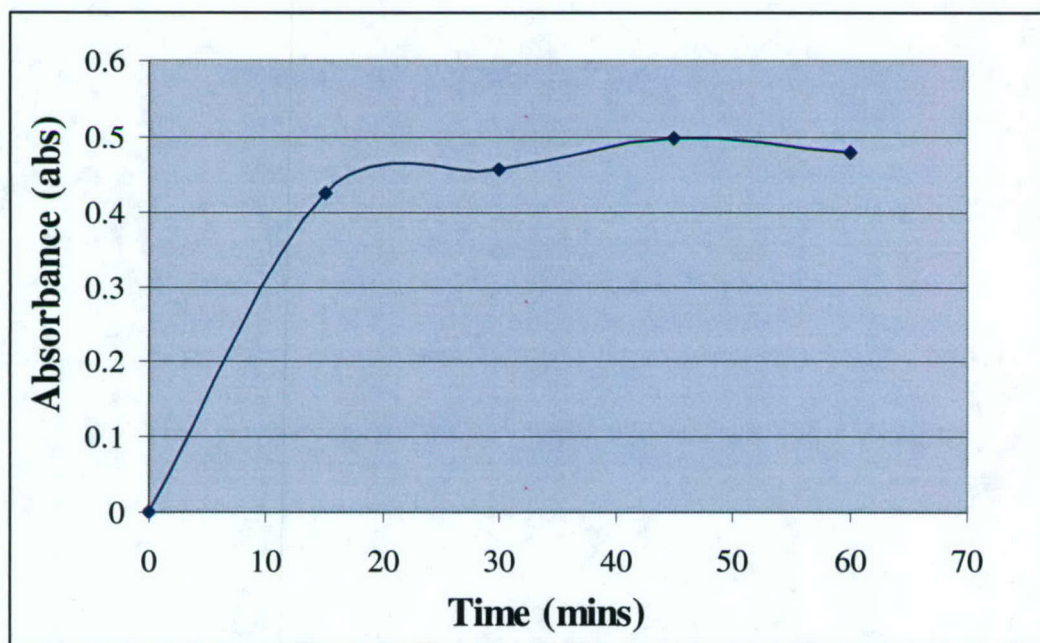


Figure 9: Release Profiles of Ampicillin from PPY in Tris-Buffered Saline

A test of the antibiotic efficacy of released amp from PPY/amp was conducted with preliminary results showing bacteria growth being inhibited in the presence of an ampicillin release solution. The study was conducted to test the bioactivity of the ampicillin following its release from an electrode by applying a low voltage electrical signal. For these studies, ampicillin released from the electrode was collected in a small volume of buffered solution and added to media to grow ampicillin-responsive bacteria. This would then allow for the biological activity of the ampicillin to be determined. This is an important point since it is essential that the electro-deposition and release steps do not reduce or abolish biological activity. As seen in the Table 1 below, ampicillin released from the electrode retained its activity as measured by decreased bacterial growth when compared to untreated cells or cells treated with elution buffer only. The optical density or OD of the sample corresponds to the number of bacteria in the culture. A higher OD reading is indicative of more bacterial cells. Results from this study support the use of this method to anchor drugs to an electrode and allow for release of biologically active drugs.

Plate	Treatment	Well	Type	590 (A)
Control	LB only	A07	M	0.1
Elution sample	Amp+LB	A08	M	0.04
buffer control	Tris+LB	A09	M	0.09

Table 1: Ampicillin release data via an electrodeposited polymer

b. Active Sense and Release

i. Nitrocellulose study

Conditions for coating glass slides with nitrocellulose have been optimized, which provides a binding surface for the attachment of antibodies. The glass is first cleaned with water, ethanol, and then diethyl ether before a small amount of nitrocellulose is applied to the surface by spinning the slide at 2000 rpm. Atomic force microscopy was used to image the surface of the slide to determine the thickness and uniformity of nitrocellulose coating. As shown in the figure 10 below, spin coating nitrocellulose allows for a relatively uniform surface for antibody attachment.

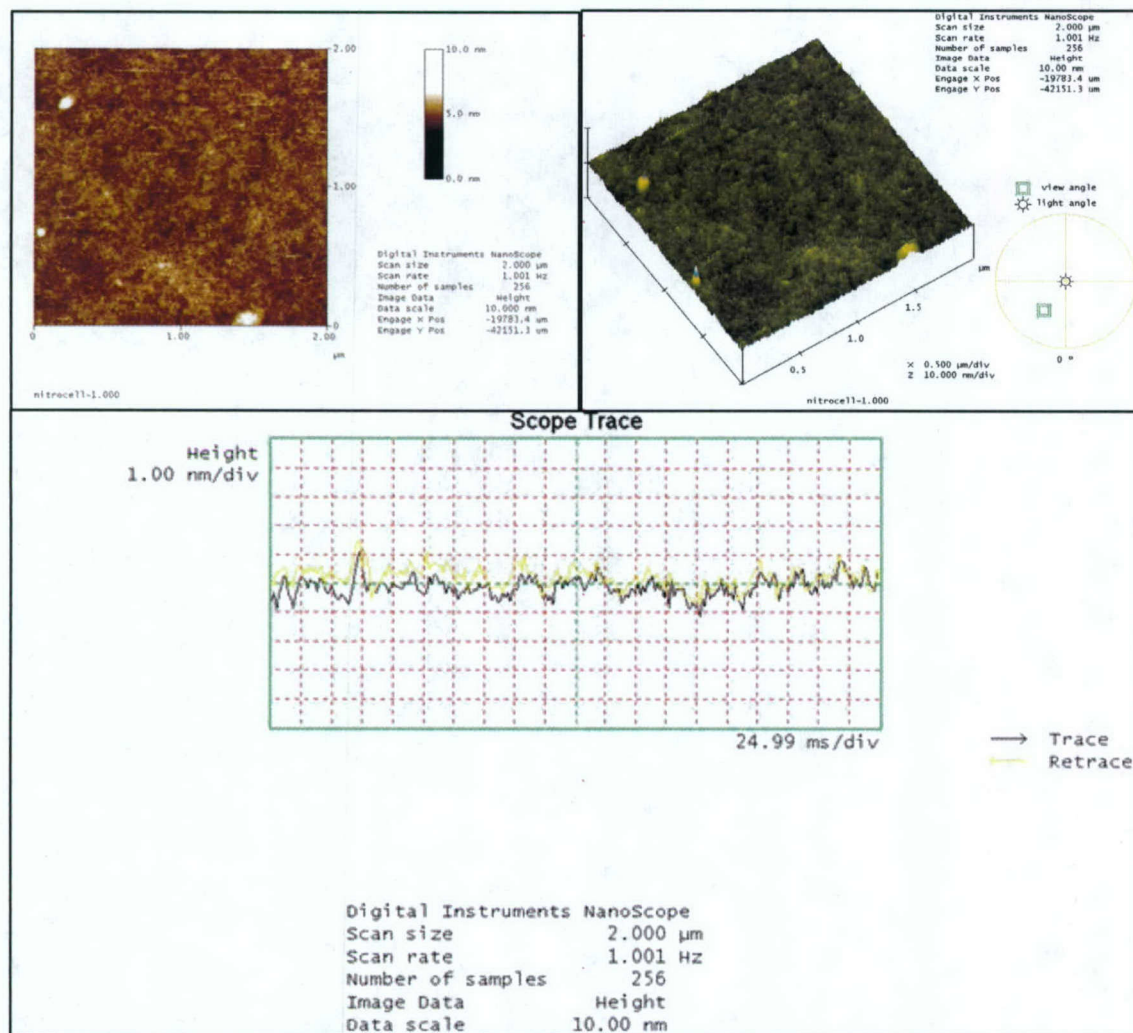


Figure 10: Top images are of topography and 3-D images of nitrocellulose respectively, and bottom is a trace of surface roughness that is less than 10nm

It was found that fluorescent antibodies would bind in a uniform manner to the nitrocellulose-coated slides. In addition it was demonstrated that antibodies that recognize E. coli bacteria can be used to capture these bacteria (see figures 11). In this experiment, bacteria was utilized that express green fluorescent protein, which allows for easy detection if bound to antibodies. Binding of the fluorescent bacteria was detected using an Olympus fluorescent microscope and photographed using a Nikon digital camera. Thus, these data provide evidence that antibodies bound to a layer of nitrocellulose on a glass slide can be used to capture bacteria in solution. Thus, we have achieved one of the main goals of this subaim to create a molecular bridge to detect the presence of bacteria in solution.

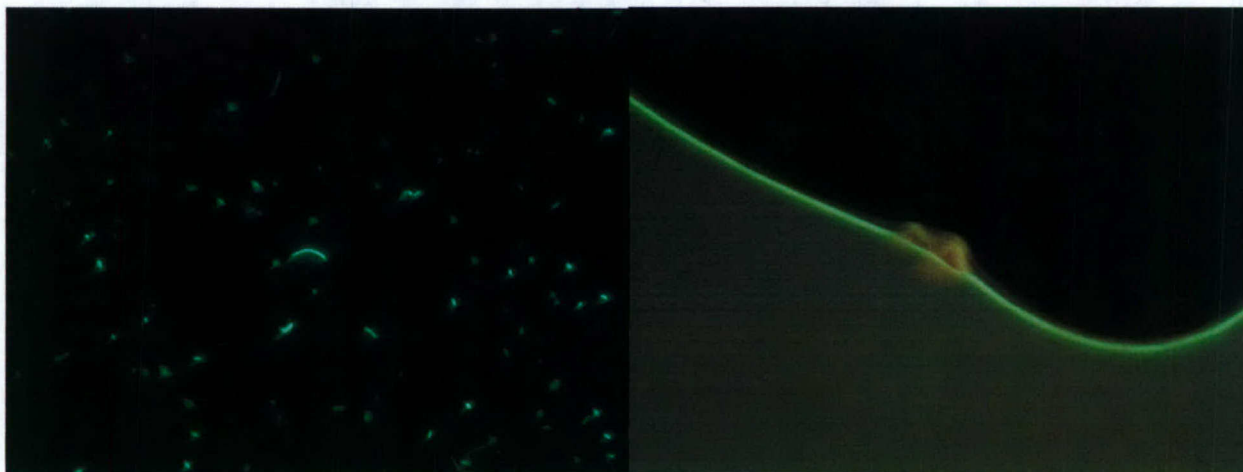


Figure 11: The image on the left is of *E. coli* bacteria captured by antibodies and the image on the right is of Primary antibody IgG binding on a NC coated membrane

i. Functionalizing of Au/PSA

The primary objective of this study is to develop an alternate method of immobilizing proteins and antibodies to the Au/PSA surface without the use of an intermediate layer such as nitrocellulose or poly-d-lysine. There are several steps used to perform the process of functionalizing the surface of Au/PSA to allow for protein or antibody binding. Initial studies for functionalizing the surface of a material to allow for antibody binding was conducted on silicon. Aldehyde silane was used as a precursor to bind onto a silicon surface to form one silane monolayer. This silane monolayer is designed for further immobilization procedures. With the reaction between the aldehyde group and the amine group of an antibody, antibodies can be immobilized onto a silicon wafer surface through chemical covalent bonding. A fluorescent microscope was used to confirm the results, and since a silicon surface can provide a relatively smooth surface at the atomic level, the AFM technique was also utilized to image antibodies on the surface.

- The silane structure we used is as followed:

11-(triethoxysilyl) undecanal

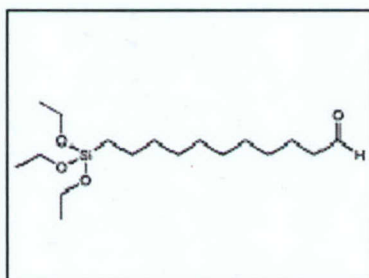


Figure 12: Silane structure

- The fabrication process is as follows:

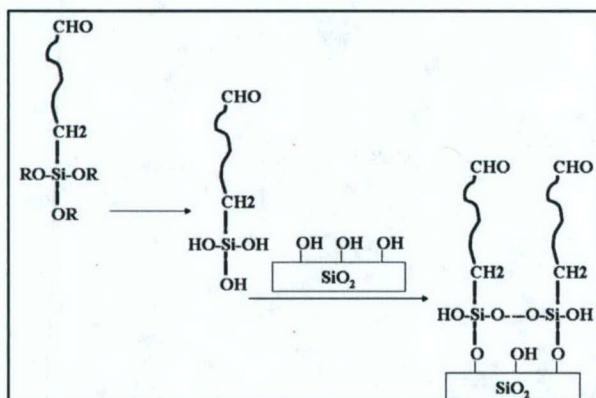


Figure 13: Immobilized antibody to silica surface by silanization

a. Immobilize antibodies on a silicon wafer surface

For the first protocol, antibody immobilization on a silicon wafer surface was conducted using the following process:

- Silicon wafer is cut to 1cm×2cm size.
- Silicon wafer is then treated with Piranha solution at 90 degree for 30mins. This causes the silicon surface to convert to silica.
- The silicon wafer is then washed with water and heated in an oven for one hour.
- The silicon wafer is then immersed in dry toluene solution contained 5%. 11 (triethoxysilyl) undecanal for 12 hours.
- After silanization is finished, the silicon wafer is washed with toluene, methanol and water, and then heated in an oven for 30mins.
- The silicon wafer with a silane monolayer is then treated with antibodies.
- Fluorescence microscopy and Atomic force microscopy is then used to characterize the immobilized antibody. (Figure 14 & 15)

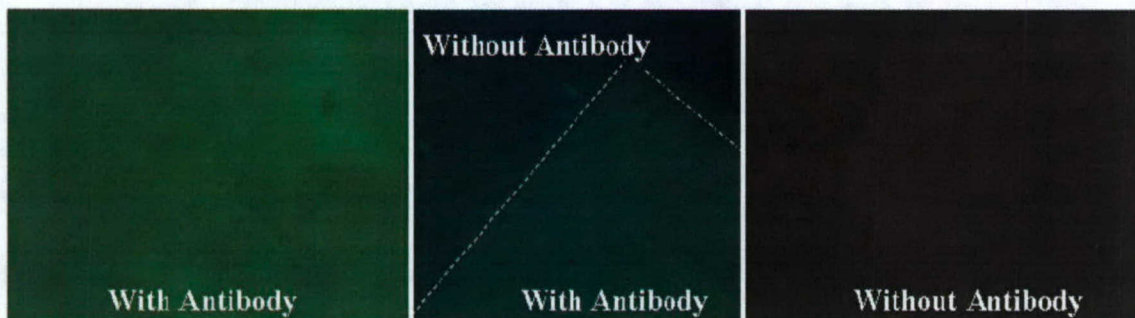


Figure 14: The Fluorescence image show the immobilized antibody on sliane surface

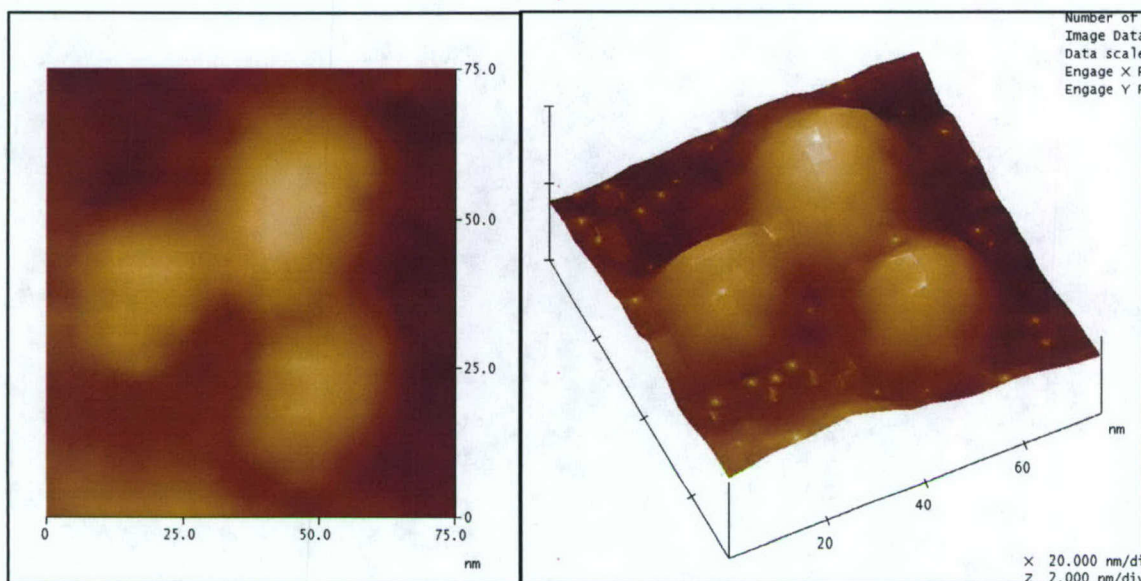


Figure 15: AFM Image of an antibody immobilized on a functionalized Si surface

b. Immobilize antibody to Au/PSA surface

Our second protocol was to immobilize antibodies onto the Au/PSA surface. The precursor for immobilization of antibodies is the Au/PSA composite. The Au/PSA surface is rich of hydroxyl groups and can be used to form a uniform silane monolayer. The aldehyde silane monolayer is formed on the Au/PSA surface through the reaction between the $-OH$ groups of the Au/PSA surface and silane. Aldehyde groups, as out layers, react with the amine groups of antibodies which causes immobilization on Au/PSA. The following list the procedure for functionalization.

- Glass slide with Au/PSA film is cut to 1cm×2cm size.
- Glass slide with Au/PSA film is treated with 1:1:5 $H_2O_2:NH_4OH:H_2O$ at 60 degree for 30 mins.
- Glass slide with Au/PSA film is then washed with water and heated in an oven for 1 hour.
- Glass slide with Au/PSA film immersed in dry toluene solution which contained 5% 11-(triethoxysilyl) undecanal for 24 hours.
- After silanization is completed the glass slide with Au/PSA film is washed with toluene, ethanol and water, and then heat in an oven for 30 mins.
- Fluorescence microscopy is used to demonstrate the immobilized antibody on the surface

Figure 16 below shows a schematic illustration of the binding of the hydroxyl groups that are formed on the Au/PSA surface with the amine groups of the antibodies. Figure 17 shows a fluorescent microscope image of antibodies on the surface of a functionalized Au/PSA material.

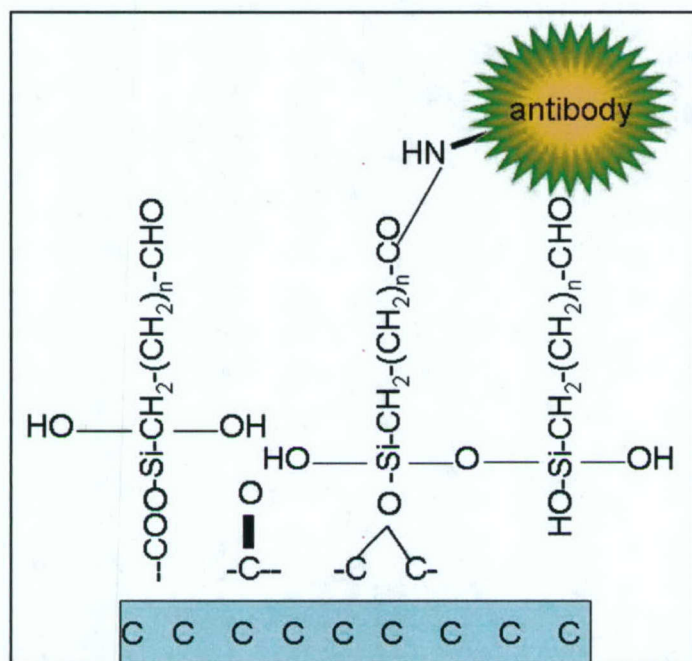


Figure 16: Immobilized antibody to Au/PSA surface by silanization

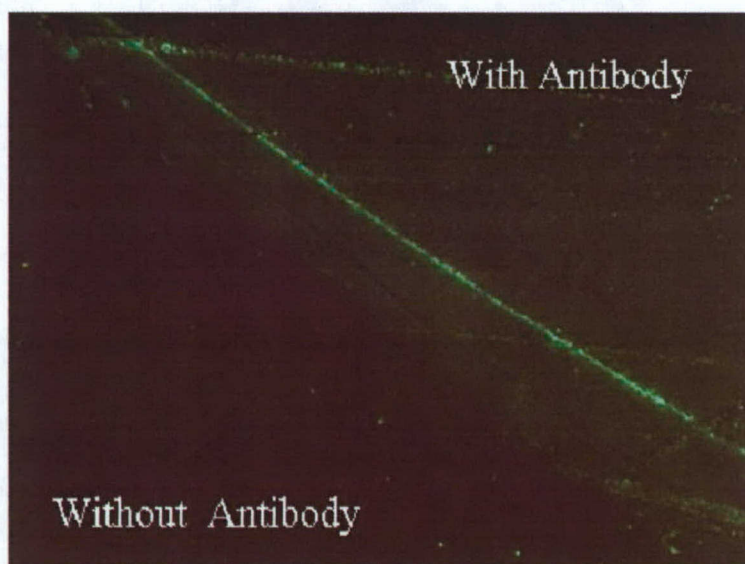


Figure 17: Fluorescence microscopy image of antibody on Au/PAS surface

We have demonstrated that it is possible to immobilize antibodies on Au/PSA surface with covalent bonding. These immobilized antibodies on the Au/PSA surface show a high stability and are suitable for our next protocol. A procedure has been developed for further work which will allow antibody immobilization on a micron sized pixel.

This procedure will include:

- The use of XPS to characterize the surface properties, before and after silanization and covalent bonding of antibodies.
- Use of contact angle techniques to characterize the surface reaction.
- Use AFM to view determine antibody image on above surface, we had got antibody image on mica surface.
- Use plasma etching technique to fabricate micro-pattern on silicon wafer and Au/PSA substrate and immobilize antibody to these patterns. This will be great help for our next step: to attach antibody to Au/PSA bridge.

c. Progress on Unity 400 Heat Dissolvable Polymer

The wet etching process used to remove SiO₂ always induces a significant amount of stress on the ion-implanted polymer bridge due to the capillary effect. It has been repeatedly reported that ion-implanted polymer bridges severely bowed after wafers were rinsed and dried. Therefore, a dry release process is highly desirable in term of device yield. As an alternative to SiO₂ sacrificial material, a thermal release polymer (Unity 400) from Promerus LLC is being investigated at BSI. At a temperature above 350°C, this material can completely decompose into a low molecular weight hydrocarbon gas that can even penetrate through an encapsulation layer, and thus a residue-free air cavity can be obtained. When the temperature ramp rate is appropriately controlled, there would be little stress on the ion-implanted polymer bridge during the thermal release process, and thus a high device yield is expected. With Unity 400 the processing steps to create CPMEMS structures are:

- 1) Deposit 1000Å WTi alloy by RF magnetron sputtering on silicon wafers that are coated with 2000Å silicon nitride;
- 2) Deposit 3000Å Au by thermal evaporation;
- 3) Deposit 1000Å WTi alloy by RF magnetron sputtering;
- 4) Spin-coat 2.5µm Unity 400;
- 5) Flash solvent at 100°C for 5 minutes;
- 6) Cure Unity 400 at 200°C for 120 minutes;
- 7) Mask wafer and perform RIE (Reactive Ion Etching) of Unity 400;
- 8) Electroplate Au pillars;
- 9) Spin-coat 1000Å PSA and implant N⁺ at a dose of 1.0x10¹⁶/cm²;
- 10) Mask wafer and perform PE (Plasma Etching) of implanted PSA;
- 11) Decompose Unity 400 at 425°C for 120 minutes;
- 12) Etch WTi/Au/WTi stack to isolate Au pillars.

The thermal decomposition kinetics of Unity 400 has been studied by using dynamic TGA (Thermal Gravimetric Analysis) at BSI. The detailed results have been shown in the previous interim report. In particular, one study revealed a strong impact of oxygen concentration on the thermal decomposition kinetics of Unity 400. In an environment of regular oxygen concentration, significant film shrinkage (~30%) was

noticed after the polymer curing process, and considerable dark residues were observed after the thermal decomposition process. Nonvolatile oxides were identified as the major constitutions of these residues.

In order to create a suitable environment for processing Unity 400, a customized tube furnace system, as shown in figure 18, was set up at BSI. Prior to processing, the quartz tube was vacuumed down to 1Torr and then continuously purged with high purity nitrogen (Grade 5). The oxygen concentration in the quartz tube was monitored in real time by a build-in oxygen analyzer. The nitrogen flow rate was adjusted so that the residual oxygen concentration in the quartz tube was less than 5ppm. In such a condition, the Unity 400 film shrinkage was less than 0.5% after the polymer curing process, and no residue could be visually noticed after the thermal decomposition process.

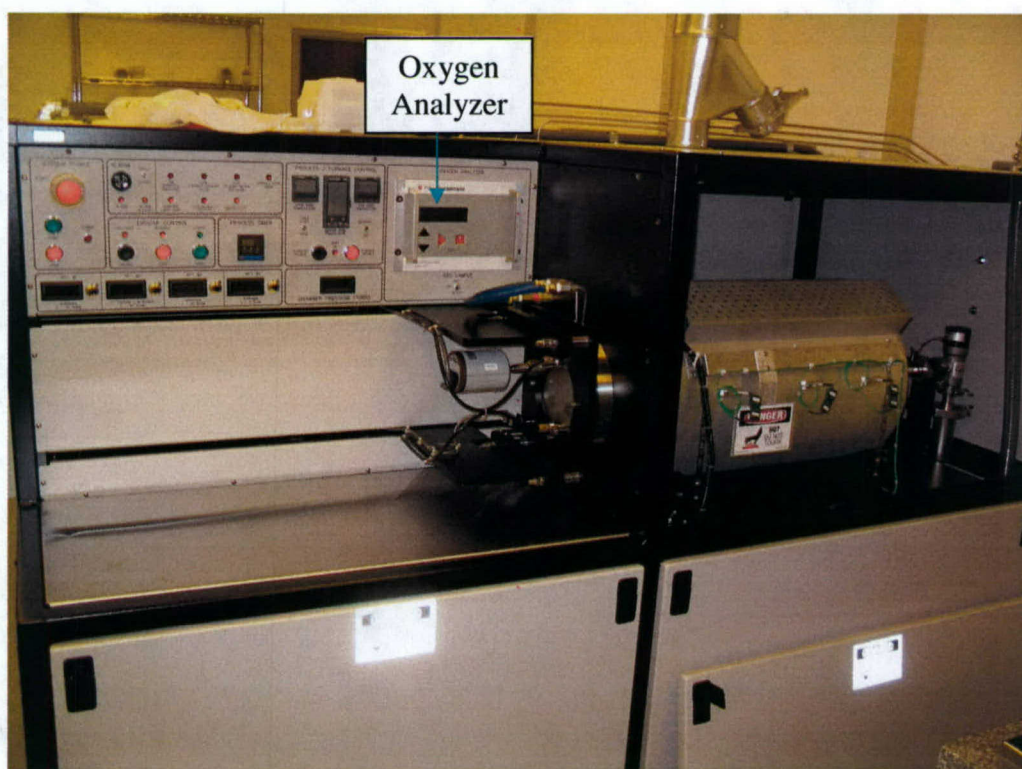


Figure 18: Tube Furnace System at BSI

The compatibility of the cured Unity 400 films in the Au electroplating bath (Aurall 305) was tested. Several silicon wafers coated with 2.5 μ m cured Unity 400 films were separately immersed in the Au electroplating bath at 60°C for as long as 65 minutes. No dissolution of these Unity 400 films or change of the Au electroplating bath were observed. These wafers were rinsed with DI water, baked at 115°C for 2 minutes, and then loaded into the tube furnace. No residue could be visually noticed after the thermal decomposition process. The results herein verified the compatibility of the cured Unity 400 films in the Au electroplating bath.

Another crucial process being investigated at BSI is step 7, which is consisted of the following sequence:

- Deposit 1000Å Al as hard mask by thermal evaporation;
- Spin-coat photoresist Shipley 1813 at 4000rpm for 60 seconds;
- Pre-bake at 115°C for 150 seconds;
- Perform UV exposure at 25mW/cm² for 4 seconds on mask aligner;
- Develop for 15 seconds;
- Post-bake at 115°C for 150 seconds;
- Perform wet etch of Al for 100 seconds to transfer photoresist pattern to Al;
- Strip photoresist Shipley 1813 with acetone;
- Perform RIE of Unity 400 to transfer Al pattern to Unity 400 layer;
- Strip Al hard mask with Al etchant.

RIE is a well-established technology in the semiconductor industry for high resolution etching of polymeric dielectrics. RIE of hydrocarbon polymers is usually performed in O₂ plasma and forms volatile but thermodynamically stable products such as CO, CO₂, and H₂O. Because Unity 400 contains silicon, nonvolatile silicon oxide can also be formed during the etching process in O₂ plasma. The accumulation of this silicon oxide layer on the polymer surface can slow down or even stop the etching process. The removal of the resulted silicon oxide layer can be achieved by adding a small amount of fluorine containing molecules into the O₂ plasma. Moreover, the concentration of the fluorine in the O₂ plasma plays an important role on the smoothness of the etched polymer surface. The sidewall profile of the etched features is highly dependent on the ratio of the physical etching to the chemical etching during the RIE process. The physical etching can be enhanced by adding Ar into the O₂ plasma.

In the previous interim report, the detailed results of the preliminary efforts on patterning Unity 400 films by RIE were shown. The major issue encountered therein was considerable undercut due to the dominance of the isotropic etching. In order to solve this problem, a new RIE system with greater capability of anisotropic etching is being set up at BSI. Meanwhile, an alternative processing sequence that does not involve direct patterning of Unity 400 films is being investigated at BSI, which consists of the following steps:

- A1) Deposit 1000Å WTi alloy by RF magnetron sputtering on silicon wafers that are coated with 2000Å silicon nitride;
- A2) Deposit 3000Å Au by thermal evaporation;
- A3) Deposit 1000Å WTi alloy by RF magnetron sputtering;
- A4) Spin-coat 4.6µm photoresist AZ 9260;
- A5) Perform photolithography to obtain pattern as electroplating mold;
- A6) Electroplate Au pillars;
- A7) Strip photoresist AZ 9260 with acetone;
- A8) Spin-coat 2.5µm Unity 400;
- A9) Spin-coat 1000Å PSA and implant N⁺ at a dose of 1.0x10¹⁶/cm²;

- A10) Mask wafer and perform PE (Plasma Etching) of implanted PSA;
- A11) Decompose Unity 400 at 425°C for 120 minutes;
- A12) Etch WTi/Au/WTi stack to isolate Au pillars.

AZ 9260 from AZ Electronic Materials is designed for the demanding high-resolution thick photoresist requirements. This photoresist provides superior aspect ratios, wide focus and exposure latitudes, and fine sidewall profiles. Two viscosity grades are available for single-coat film thickness up to 24 μ m. Critical dimension resolutions on silicon wafers by using standard broadband exposure tools range from <1 μ m lines and spaces at a film thickness of 4.6 μ m to 3.5 μ m lines and spaces at a film thickness of 24 μ m. The optimal photolithography parameters, such as exposure intensity, exposure time, development time, and so on, are being investigated at BSI. Descent results have been achieved by using the mask aligner at BSI. One typical photoresist pattern observed with SEM was shown in figure 19.

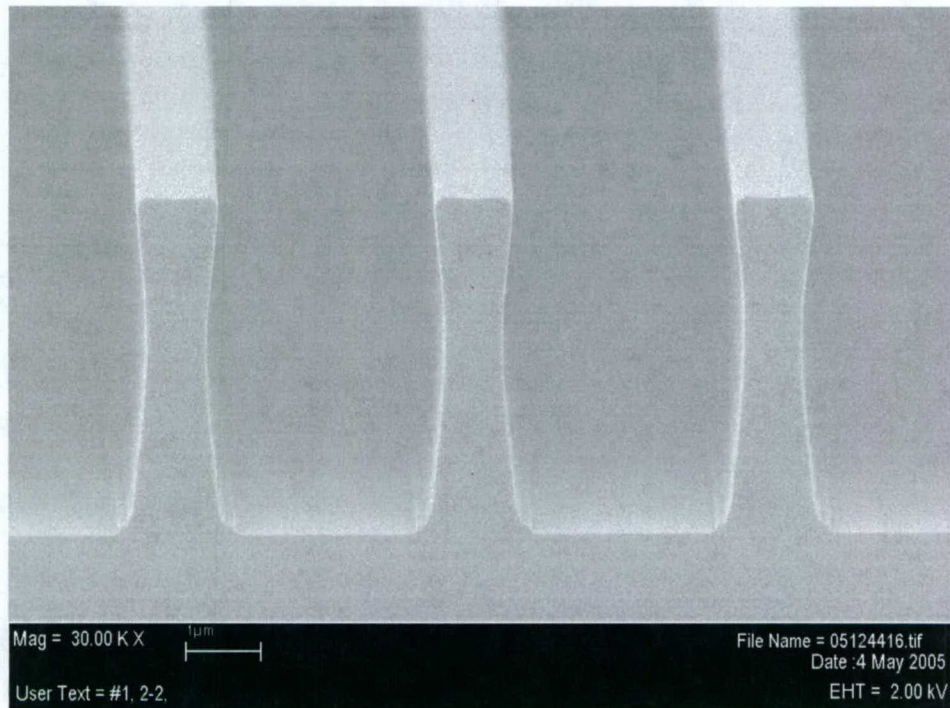


Figure 19: AZ 9260 Photoresist Pattern L/S=2 μ m/2 μ m

In figure 19 the thickness of the AZ 9260 photoresist layer was 4.6 μ m. The original features on the mask consist of 2 μ m lines and 2 μ m spaces. As shown in this SEM photo, high aspect ratio was well maintained, and the sidewall angle was fairly close to 90°. The line shrinkage at each L/S boundary was less than 0.5 μ m. The results obtained herein satisfy the requirements as the Au electroplating molds.

III. Conclusions

a. Accomplishments and Future Work

During the time frame that this interim report covers we have:

1. Successfully demonstrated a release system of Polypyrrole/phenolred on both a macro and micro scale.
2. Demonstrated the growth of a thin film of PPY/dopant on a microelectrode using two different polymerization techniques; chronoamperometry and cyclic voltammetry.
3. Established a new multi-microelectrode design for testing of the release system on a micron scale.
4. Demonstrated and tested the bioactivity of an electrodeposited antibiotic drug (Ampicillin) as a model biological active dopant.
5. Optimized conditions for spin coating nitrocellulose on glass slides for uniformity, thickness, and antibody binding efficacy.
6. Begun a study of utilizing a materials surface modification (functionalization) procedure to bind antibodies directly to the CPMEMS (Au/PSA) material without any intermediate layer such as poly-d-lysine or nitrocellulose.
7. Demonstrated the functionalization procedure on a plain Si wafer, where antibody binding has been verified with both fluorescence and atomic force microscopy.
8. Modified the functionalization procedure for Si, such that it can be applied to the Au/PSA material, where verification of antibody binding has been done via fluorescence microscopy.
9. Created a suitable environment for processing Unity 400 via a customized tube furnace system for developing the CPMEMS bridge structure.
10. Tested the compatibility of cured Unity 400 films in a Au electroplating bath, which resulted in no dissolution nor change in the Au electroplating bath.

In the months ahead, our work will include:

1. Studying the release profiles of phenolred from PPY/phenolred from the microelectrodes.

2. Coating multi-microelectrodes with different PPY/dopant.
3. Optimizing the polymerization conditions to provide enough releasable ampicillin in PPY/amp film.
4. Coating the PPY/amp surface with another protecting polymer to prevent the spontaneous release of amp in PPY/amp.
5. Investigation of an alternate biologically active dopant molecule for release experiments and conduct further tests on the biological activity of the drug released from the electrode.
6. Incorporation of a green fluorescent protein into the PPY/dopant film and the characterization of the release.
7. Begin the optimization of spin coating nitrocellulose on Si wafers and the subsequent patterning of the thin film via photolithography.
8. Conduct a study on the concentration of antibody deposition on nitrocellulose to determine the most viable detection sensitivity for CPMEMS sensor.
9. A study on the ion implantation of nitrocellulose to determine the change in its mechanical as well as antibody binding properties.
10. A further investigation into the functionalization of Au/PSA to control antibody binding such that the detection sensitivity of the CPMEMS sensor.
11. Continuation of the investigation of Unity 400 by employing a new RIE system with greater capability of anisotropic etching.

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 29-07-2005		2. REPORT DATE Interim		3. DATES COVERED (From - To) Jan 2005 – July 2005	
4. TITLE AND SUBTITLE Multiple Strategy Bio-Detection Sensor Platforms Made from Carbon and Polymer Materials				5a. CONTRACT NUMBER N/A	
				5b. GRANT NUMBER N00014-03-1-0893	
				5c. PROGRAM ELEMENT NUMBER N/A	
6. AUTHOR(S) Giedd, Ryan, Ph.D. Ghosh, Kartik, Ph.D. Curry, Matthew, M.S. Patel, Rishi, M.S. Durham, Paul, Ph.D. Kinlen, Pat, Ph.D. Han, Xuliang, Ph.D.				5d. PROJECT NUMBER N/A	
				5e. TASK NUMBER I, II, III, IV	
				5f. WORK UNIT NUMBER N/A	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Southwest Missouri State University 901 South National Avenue Springfield, MO 65804				8. PERFORMING ORGANIZATION REPORT NUMBER #4	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Regional Office Chicago 230 South Dearborn, Room 380 Chicago, IL 60604-1595				10. SPONSOR/MONITOR'S ACRONYM(S) ONR	
				11. SPONSORING/MONITORING AGENCY REPORT NUMBER N/A	
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution is Unlimited					
13. SUPPLEMENTARY NOTES Research in collaboration with Brewer Science, Inc. (Rolla, MO) and Crosslink (St. Louis, MO)					
14. ABSTRACT The goal of this project is to develop a new class of biosensors using carbon and polymer based micro-electromechanical systems (CPMEMS) and a platform that incorporates multiple bio-detection strategies. This progress report covers the development of a sense and release experiment, and further a proof of concept of actual drug release on a release experiment. Other points of progress include studies on alternate methods of developing the self assembled protein layers, specifically utilizing a nitrocellulose thin film layers or a silane functionalizing monolayer to bind proteins, antibodies or oligonucleotides to the CPMEMS sensor platform. Progress on an alternate sacrificial layer (Unity 400) to develop the CPMEMS bridge structures will also be discussed in this report.					
15. SUBJECT TERMS CPMEMS, Unity 400 Thermal Release Polymer, Active Sense and Release, Ampicillin Drug Release, Nitrocellulose, Silane Functionalization.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 23	19a. NAME OF RESPONSIBLE PERSON Dr. Ryan Giedd
a. REPORT N/A	b. ABSTRACT N/A	c. THIS PAGE N/A			19b. TELEPHONE NUMBER (Include area code) (417) 836-5279